PHYSICAL ENVIRONMENTAL AND BIOLOGICAL CORRELATES OF OTOLITH CHEMISTRY OF ARCTIC MARINE FISHES IN THE CHUKCHI SEA

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Abstract

Life history movement patterns in marine fishes can be determined by otolith chemistry if environmental variables are reflected in the otoliths. Arctic cod (*Boreogadus saida*), Arctic staghorn sculpin (*Gymnocanthus tricuspis*), and Bering flounder (*Hippoglossoides robustus*) are abundant Arctic fishes in the Chukchi Sea with overlapping distributions. Physical environmental data, demersal fishes, bottom seawater, and sediment interface seawater samples were collected from the Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) cruise on July 30, 2009 and the Russian American Long-term Census of the Arctic (RUSALCA) cruise from September 3 to 30, 2009 in the Chukchi Sea. Magnesium (Mg), strontium (Sr), barium (Ba), and calcium (Ca) were measured with an inductively coupled plasma mass spectrometer (ICP-MS) on the most recent growth edge of otoliths and in whole fish blood, as well as Ba in bottom and sediment interface seawater. Environmental variables and fish age correlated with Arctic cod and Arctic staghorn sculpin otolith signatures while only environmental variables correlated with Bering flounder signatures. Elemental correlations were not always consistent for the variables tested among species. The complexity of this multi-element tool suggests otolith chemistry may not be useful to determine life history movement patterns of these demersal Arctic fishes in offshore waters.

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1. Introduction

Fish otoliths (ear bones) are valuable natural tags to understand movement patterns and habitat for life history stages of fish populations. The microstructure of otolith growth rings contribute to the ability to assign age and growth rates to fish (Campana 2001). Over the last three decades, this chemically and temporally inert timekeeper has sparked interest in addressing questions regarding the chronology of fish life history (Elsdon et al. 2008). Elements such as magnesium (Mg), strontium (Sr), and barium (Ba) are paleoceanographic tools that reflect environmental conditions in biogenic calcium carbonates ($CaCO_3$) in otoliths, corals, and molluscan structures. These structures have been used to reconstruct past oceanic environmental conditions from daily to geological timescales.

Otolith chemical signatures can be used to discriminate groups of fishes that occupy different environmental conditions for part of their lives if two assumptions are met (Campana 1999), though fish biology and physiology may complicate the interpretations (Kalish 1989, 1991). These assumptions include 1) accreted material must remain chemically inert and not be subject to resorption, and 2) the environmental factors should influence the elemental incorporation in the otolith (Campana 1999). However, components of fish biology e.g., fish species (Gillanders and Kingsford 2003, Hamer and Jenkins 2007), growth rate (Sadovy and Severin 1994, Hamer and Jenkins 2007), and physiology, e.g., elemental regulation in blood and endolymph fluid (Kalish 1991), can

add complexities to the entrainment of elements into otoliths. For example, co-occurring species may exhibit different chemical signatures at the same environmental conditions (Hamer and Jenkins 2007, DiMaria et al. 2010). Thus, environmental and biological variables need to be validated in the otolith elemental signatures before interpretations can be made (Walther et al. 2010). In this study, I investigated the Sr, Mg, and Ba chemical signatures in the otoliths of Arctic marine fishes living in the offshore environment. This suite of elements have met the environmental assumptions (i.e., being representative of seawater conditions surrounding fishes) in past otolith chemistry investigations (Campana 1999) but are also influenced by fish biology (i.e., diet, growth rate, and age) (Kalish 1991, Sadovy and Severin 1994, Walther et al. 2010).

Aragonite otoliths daily accrete $CaCO_3$ and trace elements on a protein matrix onto the otolith surface (Campana 1999, Elsdon et al. 2008). Otoliths are situated in the inner ear of teleost fishes and reside in a membrane filled with endolymph fluid. Ambient seawater and dietary trace metal cations enter the body of marine fishes via gills and absorption into the bloodstream through intestinal digestion and are then transported to the endolymph fluid in the inner ear cavity (Campana 1999, Walther and Thorrold 2006). Trace metals and $CaCO_3$ precipitate out of the fluid and are made available for otolith biomineralization on the growing otolith edge (Campana 1999, Walther and Thorrold 2006). Magnesium has a smaller ionic radius than Ca and is thought to be incorporated into the crystal lattice at defect sites (Sinclair 2005, Hamer and Jenkins 2007). Strontium and Ba may substitute directly for Ca in crystal lattices because the ionic radii of these

two elements are larger than Ca (Speer 1983, deVries et al. 2005). Barium has the largest ionic radius compared to Ca and Sr; therefore, Ba can attach at crystal defect sites as well (Lea and Spero 1992, Campana 1999). The entrainment of elements into the otolith depends on the availability of the elements in the surrounding environment and their physiological regulation and partitioning in the fish.

Biomineralization of otolith aragonite differs from other calcified structures, such as coral or molluscan shells, as the otoliths are not exposed directly to the ambient seawater chemistry (Campana 1999). A series of physiological barriers may dilute or concentrate each element differently from ambient seawater. The intestine-seawater interface is the largest barrier to elemental uptake as marine fishes are hypotonic to seawater and work to reduce excess ions in their body. Out of Mg, Sr, and Ba, Sr is the best-studied element in otolith chemistry research (Campana 1999), which is insightful to determine potential factors affecting the incorporation of elements into otoliths. Biological and physiological factors such as pH (Gauldie et al. 1995), seasonality of gonad development, age, and gender tightly regulate the endolymph and blood plasma element chemistry (Kalish 1989, 1991), ultimately influencing the crystallization of elements into otoliths. Stressful environments (i.e., low salinity and high temperatures) influence binding, movement, and quantity of ions entrained in blood and endolymph fluid, which then can produce an elevated Sr concentration in Australian salmon (*Arripis trutta*) otoliths (Kalish 1992). Growth rate can be the primary factor affecting Sr/Ca in otoliths of fishes from different locations and temperature regimes (Sadovy and Severin 1992, 1994). Individual marine

species may have unique biology, physiology, and life history movement patterns that can further complicate the incorporation of elements onto the growing otolith surface.

Ambient environmental variables, such as temperature (Thresher 1999, Elsdon et al. 2008, DiMaria et al. 2010), salinity (Elsdon and Gillanders 2002, Martin and Thorrold 2005), and chemical concentrations in seawater (Bath et al. 2000, Hamer and Jenkins 2007) can be correlated to Mg/Ca, Sr/Ca, and Ba/Ca in fish otoliths. Concentrations of conservative elements, such as Sr and Mg, in seawater have influenced otolith chemistry in laboratory studies (Campana 1999, Elsdon et al. 2008), but have been reported as being weakly correlated or non-significant in marine field studies (Hamer and Jenkins 2007). Because Mg, Sr, and Ca are at a constant ratio to salinity in seawater, it is unlikely that marine field studies would show correlations of otolith element to Ca ratios to these ambient conservative elements in seawater. Non-conservative concentrations of elements in seawater that vary with depth and on a geographic scale, such as Ba, may influence Ba/Ca in otoliths of marine fishes (Bath et al. 2000, Hamer and Jenkins 2007). Environmental variables that influence otolith chemistry may be different within and among fish species regardless if the fishes co-exist in the same biome. Negative, positive, and no effects have been reported from field and laboratory experiments involving temperature, salinity, and chemical variables in ambient seawater and otolith signatures of these three elements (Campana 1999). Before interpretations can be made for multi-element fingerprints, otolith chemistry correlations to ambient field conditions need to be established for individual species.

The Chukchi Sea is a marginal sea to the Arctic Ocean typically consisting of at least three major bottom water masses (Figure 1). This continental shelf sea is relatively

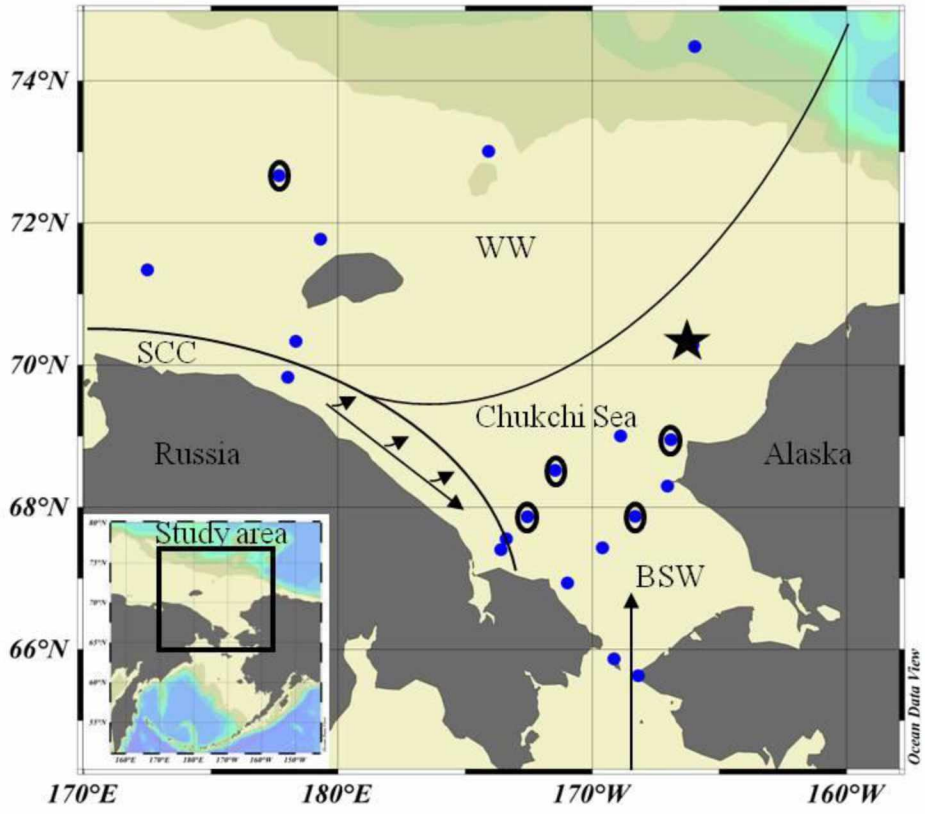

Figure 1. Study area in the Chukchi Sea where biological, physical, and chemical samples were collected at 20 stations on July 30 during COMIDA and September 3 to 30 during RUSALCA in 2009. RUSALCA stations are displayed as blue dots while the COMIDA station is the black star. Sediment interface seawater was collected at five stations (black circles). Three major water masses were detected: Bering Sea Water (BSW), Winter Water (WW), and the Siberian Coastal Current (SCC). Arrows indicate general flow patterns of water masses.

shallow (approximately 50 m)and flat-bottomed (Weingartner 1997). There is a general northward flow of currents due to the drop in sea level from the Pacific to the Arctic Ocean (Coachman et al. 1975, Weingartner et al. 1998). Bottom water masses are characterized by specific ranges of temperature, salinity, and density. The Bering Sea Water (BSW) is a warm, saline, and nutrient-rich water mass that advects northwards through the Bering Strait and into the central Chukchi Sea (Weingartner 1997). The Siberian Coastal Current (SCC), is cold, low-salinity, and nutrient-poor water that flows southward from the East Siberian Sea and into the western Chukchi Sea (Weingartner 1997). The Winter Water (WW) is a very cold and saline water mass in the central and northern Chukchi Sea (Pickart et al. 2010) formed by remnant water from the previous winter (Coachman et al. 1975). During mid-July through September, bottom water masses have relatively stable conditions compared to surface water masses that are exposed to more solar radiation, riverine input, wind mixing, and ice formation transformations.

Arctic cod (*Boreogadus saida*, Gadidae), Arctic staghorn sculpin (*Gymnocanthus tricuspis*, Cottidae), and Bering flounder (*Hippoglossoides robustus*, Pleuronectidae) are demersal (live near seafloor) Arctic fishes that are abundant in the Chukchi Sea and have varying geographical ranges (Mecklenburg et al. 2002, 2007). These species serve as prey for apex predators, such as pinnipeds and seabirds, and are ecologically important in the Arctic benthic food web (Frost and Lowry 1980, Springer et al. 1987, Bluhm and Gradinger 2008). Arctic cod, a circumpolar species, has the largest geographical

distribution of the three species from river mouths to depths down to 731 m offshore in Arctic basin waters (Mecklenburg et al. 2002). Arctic staghorn sculpin have been found throughout the Chukchi Sea from depths of 7-240 m (Mecklenburg et al. 2002). Bering flounder are limited by a northern range restriction into the Chukchi Sea and are found at depths of 18-425 m (Mecklenburg et al. 2002). Spawning of all three species occurs during winter months in nearshore environments (Craig et al. 1982, National Research Council 1996, Smith et al. 1997). All three species have a pelagic larval stage and then settle to the benthos to complete the remaining life history stages (Wyllie-Echeverria et al. 1997, Norcross et al. 2010). Once settled in the demersal environment, Arctic staghorn sculpin and Bering flounder remain near the seafloor for the duration of their lives whereas Arctic cod can occupy demersal, pelagic, and cryopelagic (ice-associated) zones in coastal and offshore marine habitats (Craig et al. 1982, Bluhm and Gradinger 2008, Norcross et al. 2010).

Barium is a non-conservative element in seawater that is used as a chemical tracer of water masses receiving fluvial input in the Chukchi Sea (Guay and Falkner 1997). Barium is a biointermediate element that has a nutrient-type vertical profile in the marine environment (Guay and Falkner 1997). Elevated concentrations of Ba in surface seawater occur from fluvial input (Guay and Falkner 1997, Cooper et al. 2008, Abrahamsen et al. 2009). In comparison to WW and the offshore waters of BSW in the Chukchi Sea, elevated levels of Ba are expected in SCC surface waters, which drain the river runoff in the East Siberian Sea. In biologically productive surface seawater,

dissolved Ba forms barite ($BaSO_4$) and is exported in association with sinking biological particulate matter, which causes enrichment and possible regeneration at depth and in sediments (Dehairs et al. 1980, Guay and Falkner 1997, Abrahamsen et al. 2009). Barium enrichment at depth due to high productivity in overlying surface waters has been documented near Wrangell Island in the western Chukchi Sea (Guay and Falkner 1997, Abrahamsen et al. 2009). If ambient seawater Ba is reflected in otolith signatures, preference of Arctic marine fishes for specific bottom water masses, and movement patterns between nearshore and offshore habitats, can potentially be tracked.

In the Chukchi Sea, otolith chemistry can serve as a potential tool to reconstruct the occupation of bottom water masses by demersal fishes if otoliths reflect physical characteristics associated with water masses. Water temperature and salinity effects on Mg/Ca, Sr/Ca, and Ba/Ca in otoliths have allowed for distinctions to be made among treatment groups of fishes in laboratory experiments (Elsdon and Gillanders 2002), thus this suite of elements could be utilized as a tracer of marine fishes among water masses. Otolith edge signatures of Mg/Ca, Sr/Ca, and Ba/Ca in oceanic fishes have been used to discriminate frontal zones and water mass occupation of fishes in the Antarctic Circumpolar Current in the Southwestern Atlantic Ocean (Ashford et al. 2007). Arctic fishes community assemblages are associated with specific bottom water masses, i.e., WW and BSW, in the Chukchi Sea (Norcross et al. 2010), with bottom salinity and sediment type as the primary environmental factors contributing to fish community structures (Barber et al. 1997, Norcross et al. 2010). Benthic hotspots with high biomass,

located in the eastern and northeastern Chukchi Sea, have been persistent through time from the early 1970s to 2004 (Grebmeier et al. 2006) and these hotspots may be traceable with Ba measured in either bottom seawater or sediment interface seawater (pore water from 0-2 cm depth of surface sediment) due to the tight benthic-pelagic coupling characteristic of the Chukchi Sea. The demersal fishes in this study may correlate with the Ba concentrations in the bottom seawater and sediment interface seawater because these fishes live near the seafloor and rest on sediment. Barium may be particularly useful as an environmental marker in otoliths of the three Arctic fishes in this study, tracing fish movement among bottom water masses and their proximity to benthic hotspots in this region. Thus, Mg, Sr, and Ba may serve as a multi-element tool to discriminate assemblages of fishes among water masses on a mesoscale in Arctic waters.

The purpose of this study is to determine if individual elemental concentrations on the recent edge (Figure 2) of otoliths of demersal fishes reflects the physical environmental (i.e., ambient concentrations of Ba in bottom and sediment interface seawater, temperature, and salinity), biological (i.e., fish species, age of fish), or physiological variables (Mg, Sr, and Ba in whole fish blood). Otolith chemistry is becoming more prevalent in fisheries research (Campana 1999, Campana and Thorrold 2001), though applied studies investigating which primary factors influence otolith chemical signatures are lacking (Walther and Thorrold 2006, Walther et al. 2010). I hypothesized that otolith chemistry of Arctic cod, Arctic staghorn sculpin, and Bering flounder reflects the unique

physical environmental characteristics of bottom water masses in the Chukchi Sea, while biological and physiological factors are of only minor importance to Mg/Ca, Sr/Ca, and

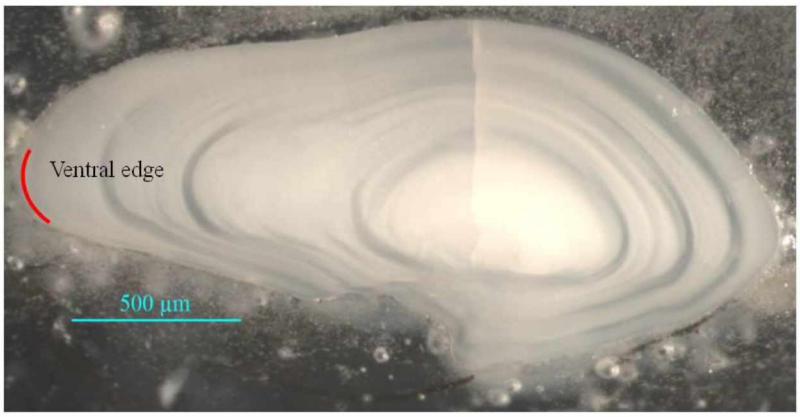

Figure 2. Laser ablation sample location labeled on an Arctic staghorn sculpin thin-sectioned otolith (age-3). Otoliths were ablated with a continuous 25 µm wide line on the ventral edge (red line).

Ba/Ca otolith signatures. Arctic fish communities (which include these three fish species) in the Chukchi Sea are structured by bottom water masses (Norcross et al. 2010), which have distinct water temperature, salinity, and density characteristics; therefore, these marine fishes are living in a stable, yet unique set of environmental conditions. Over the short duration of this study (about 1 month) during the fall, I assume the bottom water mass variables do not shift drastically. I am also assuming that the area analyzed on the otoliths was formed while the fishes inhabited one water mass and movement between water masses was minimal.

2. Methods

2.1 Sample collection

Demersal Arctic fishes and seawater samples were collected simultaneously aboard multidisciplinary research cruises in fall 2009 in the Chukchi Sea (Figure 1). Samples were primarily collected on the Russian American Long-term Census of the Arctic (RUSALCA) research cruise from September 3 to 30, 2009 with the goal to provide mesoscale coverage via transects across the Chukchi Sea. The Chukchi Sea Offshore Monitoring in Drilling Area (COMIDA) research cruise was from July 21 to August 12, 2009 in the Bureau of Ocean Energy Management (BOEM) Lease Sale 193 Area off the northwestern shore of Alaska, one supplemental station was also sampled on July 30 was used for this study. July through September 2009 was relatively ice-free in the Chukchi Sea, although substantial areas of icebergs were encountered on both cruises, limiting sampling efforts to some extent. Samples were collected at stations with wide geographical coverage and range of temperatures and salinities.

Environmental variables (i.e., temperature, depth, salinity) were measured and bottom seawater was collected at stations approximately 1-3 m above the seafloor. Of the 20 stations where fishes were collected, 14 stations had ambient bottom seawater collected prior to fish trawls. Due to time restraints on sampling efforts, the remaining six stations required substitutions of bottom seawater samples from adjacent stations. RUSALCA was the primary cruise for seawater data collection (n = 19) using a Sea-Bird model

SBE911+ CTD profiler equipped with 10 L Niskin bottles. The COMIDA cruise provided one supplemental station where CTD data were collected with an YSI SONDE 6600 and bottom seawater was collected from a peristaltic pump with 30 m of clean Tygon tubing (4 cm diameter) attached to a Teflon weight and equipped with a HOBO pressure and temperature sensor (U20).

Bottom seawater samples were collected in acid-washed high density polyethelene (HDPE) Nalgene® bottles (125 mL, amber) that were pre-loaded with nitric acid. A simple serial dilution Equation 1,

$$C_1V_1 = C_2V_2 \quad (1)$$

was used to determine the concentration (C_n) and volume (V_n) of nitric acid to acidify samples. Clean bottles were pre-loaded with 12.5 mL of Omnitrace® 20% nitric acid for a final acidified seawater sample concentration of 1.8% (Equation 1). Bottles were filled to the brim with bottom seawater and with a final volume of 140 mL. Blank samples consisted of acid-washed and pre-loaded bottles that were filled with Milli-Q water at sea under the same atmospheric conditions at stations where bottom seawater was collected. Seawater samples were stored in coolers at sea (air temperature 0 to 10 °C) and refrigerated at 4 °C in the laboratory until analyzed at the UAF Advanced Instrumentation Laboratory (AIL).

Sediment interface seawater samples were collected from 0-2 cm of seafloor surface sediment at five fishing stations during the RUSALCA cruise. A HAPS corer was used to collect a stratified layer of seafloor sediment (15-30 cm depth) and overlying bottom seawater (0-10 cm). Bottom seawater was syringed off the top of the sediment, then a subsample of 0-2 cm depth of surface sediment was pipetted into sterile plastic 4 mL BD vacutainers® (K_2 EDTA whole blood anticoagulant). Surface sediment was centrifuged (5 min, 3,000 rpm) until pore water could be pipetted into individual vacutainers for a sample volume of 2.5 to 4 mL per station. Syringes and pipettes were disposed of after each liquid transfer to prevent contamination. Milli-Q water was poured into vacutainers at sea for field sample blanks. Sediment interface seawater was stored in coolers at sea (air temperature 0 to 10 °C) and then refrigerated at 4 °C until analyzed at the UAF AIL.

Arctic fishes were collected near the seafloor in the BSW, SCC, and WW water masses in the Chukchi Sea (Figure 3). Demersal fishes were collected with a plumb staff beam trawl with a 4 mm mesh codend liner. Details on trawling methods can be found in Norcross et al. (2010). Arctic cod (n = 128), Arctic staghorn sculpin (n = 115), and Bering flounder (n = 59) were captured during RUSALCA. Bering flounder (n = 1) were also collected during COMIDA. Whole blood was collected from 27 Arctic cod, 55 Arctic staghorn sculpin, and 27 Bering flounder after capture at sea. The tail was cut off at the caudal peduncle of the fish to extract blood from the dorsal aorta (Kalish 1991). Whole blood samples were stored in sterile plastic 4 mL BD vacutainers® (K_2 EDTA whole blood anticoagulant) and frozen at -20 °C until analyzed for trace elements at the

UAF AIL (see detailed methods in Appendix A). At sea, fishes were identified to species and frozen at -20 °C until further laboratory analyses. Fish trawl collections were in

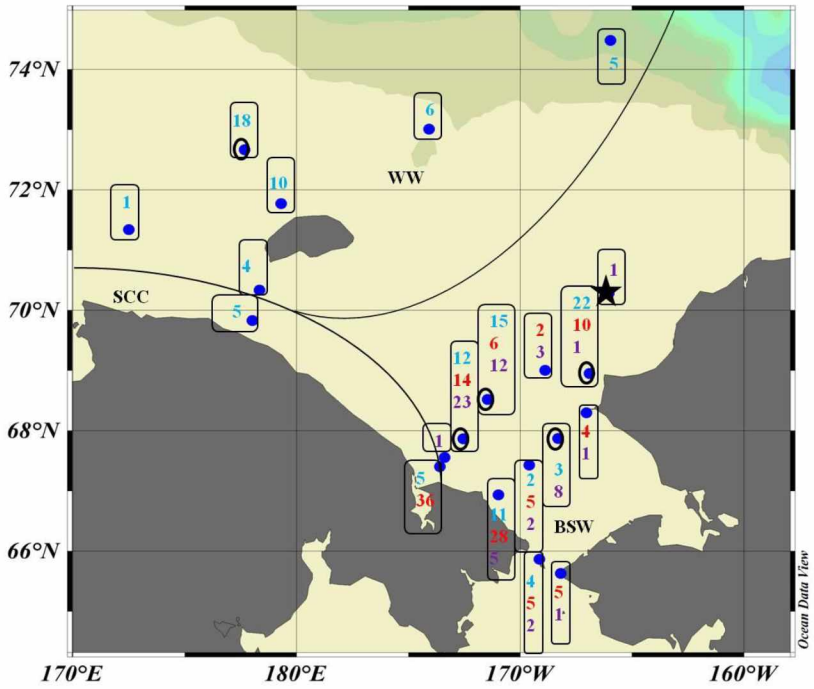

Figure 3. Sampling distributions and sample sizes by station of demersal fishes collected in the Chukchi Sea. Bottom stations from RUSALCA (blue dots) and COMIDA (black star) research cruises are displayed as well as stations where sediment interface seawater (black circles) was collected. Sample sizes are presented for each species next to the station of capture; Arctic cod (blue), Arctic staghorn sculpin (red), and Bering flounder (purple). Bering Sea Water (BSW), Winter Water (WW) and Siberian Coastal Current (SCC) are the bottom water masses and are divided by the black lines.

accordance with International Animal Care and Use Committee Protocol#: 07-47, Alaska Department of Fish and Game research permit CF-09-101, and acknowledged by the National Marine Fisheries Service (LOA 2009-08).

2.2 Otolith preparation and analyses

At the Fisheries Oceanography Laboratory at UAF, sagittal otoliths were removed from fishes and prepared for chemical analysis at the UAF AIL. To mount multiple polished otoliths onto 25 x 45 x 1.27 mm petrographic slides for efficient chemical analysis, a small piece of glass cover slip was bonded to a 25 x 76 mm slide with heated wax, then one otolith per individual (right or left otolith was randomly chosen) was mounted in heated Crystalbond™509 thermoplastic cement onto the cover slip (Dr. Christian Zimmerman (U.S. Geological Survey), personal communication). Otoliths were thin sectioned using a Buehler® isomet low speed saw, reheated to place the flat edge of the otolith onto the glass, and ground down to about 200-400 µm thickness with 5, 9, and 15 µm lapping film on a Buehler rotating wheel until growth rings were visible under a compound microscope. The second otolith per individual was prepared if the first otolith broke off during processing. A transmitted and reflected light photo of each otolith was taken with a camera-mounted dissecting microscope (Leica M165C). Otoliths from photos were aged by two independent readers by counting annual growth rings. When readers disagreed on assessment of fish age, the readers re-aged the questionable otoliths concurrently to assign a final age. Glass cover slips with a single polished otolith were reheated slightly to transfer up to nine otoliths onto a single 25 x 45 x 1.27 mm slide. Sample slides were rinsed with Milli-Q water and air dried in a slide storage box prior to chemical analysis.

A New Wave UP-213 laser ablation (LA) system coupled with an Agilent 7500ce quadrupole inductively coupled plasma mass spectrometer (ICP-MS) fitted with a cs lens stack was used to detect a suite of trace metals and macroelements in otolith samples at the UAF AIL. Calcium ($Ca^{42, 43, 44}$), magnesium (Mg^{24}), strontium (Sr^{88}), and barium (Ba^{138}) were measured in each sample. This suite of elements and isotopes were chosen for their highest natural abundances with the least interferences in the ICP-MS and were measured above the detection limits in preliminary analysis of otoliths from the three fish species. The LA-ICP-MS was cleaned and tuned at the start of each laser ablation day. Laser ablation settings were set to 80% power, 30 s warm-up, 5 s dwell time, pulse rate of 10 Hz, laser sampling rate of 10 µm/s, 5 µm sample depth, and 25 µm line width.

The standard reference materials (SRM) and edge sampling procedure in the LA-ICPMS were the same for all species and ages of fish. Each time the sample chamber was opened, a set of three SRMs was placed on the otolith slide and ablated with a 120 µm long continuous line prior to otolith ablation. Background levels of the analytes were collected for 30 s prior to each SRM and otolith sample. National Institute of Standards and Technology® (NIST) 610 glass material was used as the primary SRM in otolith chemical analysis to calibrate the otolith dataset. NIST612 (glass) and National Research Council (NRC) of Canada FEBS-1 (otolith) SRMs were used as secondary SRMs to determine the accuracy and precision of ICP-MS measurements. Otoliths were ablated with a 350-500 µm line parallel to the most recent growth ring on the ventral edge

(Figure 2) in the summer growth area (representing about 2 weeks to 1 month of accretion).

The element to Ca molar ratio was determined by a series of calculations for all otolith samples (Longerich et al. 1996). Averaged counts per second (cps) background levels were subtracted from averaged cps of each analyte measured in samples and standards. Ca^{43} was the internal standard (ISTD) used to account for instrument drift (Campana et al. 1997). Concentrations of each analyte in the sample ($C_{AN_{SAM}}$) were calculated by Equation 2,

$$C_{AN_{SAM}} = \frac{R_{AN_{SAM}}}{S} \quad (2)$$

where $R_{AN_{SAM}}$ = cps of the analyte in the sample and S = normalized sensitivity. The S term is determined by Equation 3,

$$S = \left(\frac{R_{AN_{CAL}}}{C_{AN_{CAL}}}\right) \times \left[\left(\frac{R_{IS_{SAM}}}{R_{IS_{CAL}}}\right) \times \left(\frac{C_{IS_{CAL}}}{C_{IS_{SAM}}}\right)\right] \quad (3)$$

where $R_{AN_{CAL}}$ = cps of the analyte in NIST 610, $C_{AN_{CAL}}$ = certified concentration in NIST 610, $R_{IS_{SAM}}$ = cps of the ISTD in the sample, $R_{IS_{CAL}}$ = cps of the ISTD in NIST 610, $C_{IS_{CAL}}$ = certified concentration of ISTD in NIST 610, and $C_{IS_{SAM}}$ = concentration of the ISTD in the sample. Concentrations of analytes were calculated in µg/mL and were

converted to molar concentrations by dividing the concentration (µg/mL) by the molar weight of the analyte. Analytes were divided by the molar concentration of Ca^{42} to normalize the ratios (Campana 1999). The limits of detection (LOD) of each analyte were determined by Equation 4,

$$LOD = \frac{3\sigma_{individual}}{S} \times \sqrt{\frac{1}{n_b} + \frac{1}{n_a}} \quad (4)$$

where $individual$ = averaged background cps of sample, S = normalized sensitivity, n_b = number of background measurements, and n_a = number of analyte measurements in the otolith sample. Typical LOD for otolith edge samples were Ca^{42} = 97.03, Ca^{43} = 166.69, Mg^{24} = 0.08, Sr^{88} = 0.30, Ba^{138} = 0.01 mg/kg. The percent relative error is a determination of how accurate instrument measurement values (v_{approx}) are compared to reference values (v) of the SRM using Equation 5. Percent relative errors for FEBS-1 were Ca^{42} = 4.8%, Mg^{24} = 1.7%, Sr^{88} = 11.4%, Ba^{138} = 19.2%.

$$Percent\ relative\ error = \left(\frac{|v - v_{approx}|}{|v|}\right) \times 100\% \quad (5)$$

2.3 Seawater preparation and analyses

Ambient seawater concentrations of $Ba^{137, 138}$ were measured in the bottom and sediment interface seawater using standard addition methods (Danzer 2007) with an ICP-MS equipped with an Agilent high matrix sample introduction (HMI) accessory at the UAF

AIL. ICP-MS components were cleaned and tubing was replaced after the completion of each analytical period. Cones were conditioned for about 20 min with Bering Sea seawater (28 µg/L Ba^{138}) at the appropriate dilution levels prior to analyzing Chukchi Sea seawater samples. Ultra-robust settings were used and the instrument was tuned at the beginning of each sampling day. The SRM consisted of NASS-5 (Leonhard et al. 2002) which is offshore seawater from NRC. SRMs were used to determine the accuracy and precision of the ICP-MS measurements. Seawater, blanks, and standard samples were individually filtered in the laboratory with disposable 45µm Teflon filters attached to Leur-Lock 10 mL syringes. Samples were refrigerated at 4 °C until analysis.

Bottom seawater and blank samples were analyzed as straight concentrations of samples while sediment interface seawater was prepared for a 1/10 dilution (Leonhard et al. 2002) with 2% nitric acid to obtain enough sample volume (5 mL) for analysis. The samples were split into four subsamples and each was spiked with Ba stock solution of 25, 50 or 100 µg/L for a total of three Ba level spikes and a raw seawater sample per field station. Samples were agitated and randomized prior to analysis. Analytical runs were limited to 12 hr runs to prevent build-up of material on the cones and lens stack to ensure measurement sensitivity. Diluted seawater samples were analyzed on a separate day from raw samples. $Indium^{115}$ (Hamer and Jenkins 2007) and $Germanium^{72}$ were used as ISTDs to correct measurements of Ba for instrument drift. Continuous calibration verification (CCV) at 100 and 200 µg/L Ba, continuous calibration blanks (CCB) (2% nitric acid), and NASS-5 were measured every 10 analytical samples (about every third

station of sample spike sets). Nitric acid (2%) and Milli-Q water were used as the two rinse solutions following each sample. Sample blanks (2% nitric acid) were prepared in the laboratory and preceded each set of spiked station samples.

The standard addition method (Danzer 2007) was used to back-calculate the ambient concentration of Ba in raw seawater samples by using measured spiked levels of calibration standards. External calibration lines were assessed for each isotope and gas mode to ensure R^2 values of at least 0.99. Samples were averaged for normal (no gas), hydrogen (H), and helium (He) gas modes in the reaction cell of the ICP-MS as seawater methods were in the developmental stage (Dr. Tom Trainer, (UAF) personal communication) and relative errors were less than 20% in each mode. In the ChemStation software (v. B04. 00, Agilent Technologies 1989), weights were applied to diluted sediment interface seawater samples during data processing. A dilution factor of an additional 9% was applied to measured concentrations of Ba in bottom seawater as it comprised 91% of the total sample volume in preloaded acidified field sample bottles. ChemStation software calculated the LOD for Ba, which was determined by three times the standard deviation at the zero concentration level. LOD for Ba^{138} for bottom and sediment interface seawater samples was 0.01 μg/L. Percent relative errors (Equation 5) of NASS-5 for Ba^{138} was less than 9.4% for bottom seawater analyses, while sediment interface seawater was less than 4.5%.

Calcium was calculated based on *in situ* salinity and was used to report Ba/Ca ratios for bottom seawater. Sediment interface seawater was not reported in ratio to Ca because salinity values were not attained during field sample collection, and Ca could not be measured in the samples. Conservative elements in ambient seawater are at a constant ratio to salinity and can be calculated with Equations 6 and 7,

$$Chlorinity = \frac{Salinity}{1.80655} \quad (6)$$

$$Calcium = \frac{0.02125}{Chlorinity} \quad (7)$$

(Campana 1999, Libes 2009, Dr. John Trefry (Florida Institute of Technology), personal communication). Elemental ratios (g/kg) to chlorinity are 2.13×10^{-2} for Ca, 6.68×10^{-2} Mg, and 4.10×10^{-4} Sr (Riley and Chester 1971, Libes 2009). The offshore marine environment Mg/Ca will always yield a molar ratio of 5.18 mol/mol and Sr/Ca yields 8.83 mmol/mol (Riley and Chester 1971, Libes 2009).

2.4 Statistical analyses

Element to Ca ratio means on the outer edge on otoliths were compared for interspecific species in the BSW and intraspecific species in multiple water masses with parametric methods using one-way ANOVAs ($p < 0.05$) and Tukey Honestly Significant Differences (HSD) tests ($p < 0.05$) in Sigma Plot software (v. 11.0, Systat Software Inc. 2008). Outliers, defined as > 3 standard deviations of the mean value per element and fish

species, were removed for otolith (DiMaria et al. 2010) and whole blood chemistry data. Data assumptions of independence, equal variance, and normality were satisfied prior to parametric analysis. A $\log_{10}$ transformation was applied to otolith chemistry for Mg/Ca, Sr/Ca, and Ba/Ca to satisfy normality assumptions for statistical analyses. Water masses were identified with potential density plots in Ocean Data View (ODV) (v. 4.3.5, Schlitzer 2010) using 111 stations from RUSALCA 2009. Intraspecific species variation of element to Ca ratios were compared for Arctic cod and Arctic staghorn sculpin while interspecific species variation was assessed for all three fish species in the BSW per element.

The association of physical environmental, biological, and physiological variables on otolith chemistry for each species was evaluated with partial correlations from multiple linear regression (MLR) (R statistical program v. 2.13.1, R Development Core Team 2011) and correlations from simple linear regressions (SLR) models in Sigma Plot software (v. 11.0, Systat Software Inc. 2008). Regression variables were classified as environmental (i.e., seawater temperature, salinity, Ba/Ca in bottom water, and Ba in sediment interface seawater), biological (i.e., fish age and species), and physiological (i.e., Mg/Ca, Sr/Ca, and Ba/Ca in whole blood). To meet parametric data assumptions, otolith chemistry data required $\log_{10}$ transformations to satisfy normality assumptions. Each fish species and element in the otolith was assessed with MLR models with input variables of age, temperature, and salinity. In MLR, the variance inflation factor for the model was larger than 10 when bottom seawater Ba was included; therefore, SLRs were

used to assess this variable (Dr. Arny Blanchard (UAF), personal communication). Sediment interface seawater (Ba) and blood chemistry (Mg/Ca, Sr/Ca, and Ba/Ca) samples were excluded from MLR because these variables had small samples sizes and were exploratory components to this project. Ba in sediment interface and Ba/Ca otolith correlations were assessed with SLRs. Significant regression statistics were reported ($p < 0.05$). Spearman correlations were assessed for whole fish blood chemistry. The strength of correlations (r) were described as weak ($\pm$ 0.1–0.3), moderate ($\pm$ 0.3–0.5), and strong ($\pm$ 0.5–1.0) in the text.

3. Results

3.1 Bottom water masses

During 2009, the area sampled in the Chukchi Sea had three bottom water masses, with variable physical and chemical properties (Figure 1, Table 1). Bering Sea Water (BSW) covered about half the sampling area in the Chukchi Sea. Temperatures were highest near both Russian and Alaskan coastlines (high of 4.0 °C) and decreased offshore (low of -0.1 °C) in the BSW (Figure 4a). The BSW was characterized by low variability in salinity and bottom water depth (Figure 4b). In BSW, Ba in sediment interface seawater

Table 1

Physical and chemical property means (ranges) of Chukchi Sea bottom water masses. Bottom water masses include Bering Sea Water (BSW), Siberian Coastal Current (SCC), and Winter Water (WW). Sediment interface water was collected at four stations in Bering Sea Water and one station in Winter Water. Bw = bottom water, Iw = interface water. nd = not determined

Chukchi Sea water masses	Stations (n = stations)	Depth [m]	Water temperature [˚C]	Salinity	Ba Bw [µg/L]	Ba Iw [µg/L]
BSW	12	**44.2**	**2.1**	**32.2**	**11.48**	**20.02**
		(36.6-52.5)	(-0.1-4.0)	(30.90-33.0)	(9.29-12.94)	(13.67-30.46)
SCC	2	**34.6**	**3.7**	**27.2**	**6.72**	nd
		(29.7-39.6)	(no range)	(25.8-28.7)	(5.32-8.12)	nd
WW	6	**127.8**	**-0.6**	**32.7**	**11.27**	**3.45**
		(31.7-347.0)	(-1.5-0.8)	(31.7-34.8)	(11.15-11.80)	nd

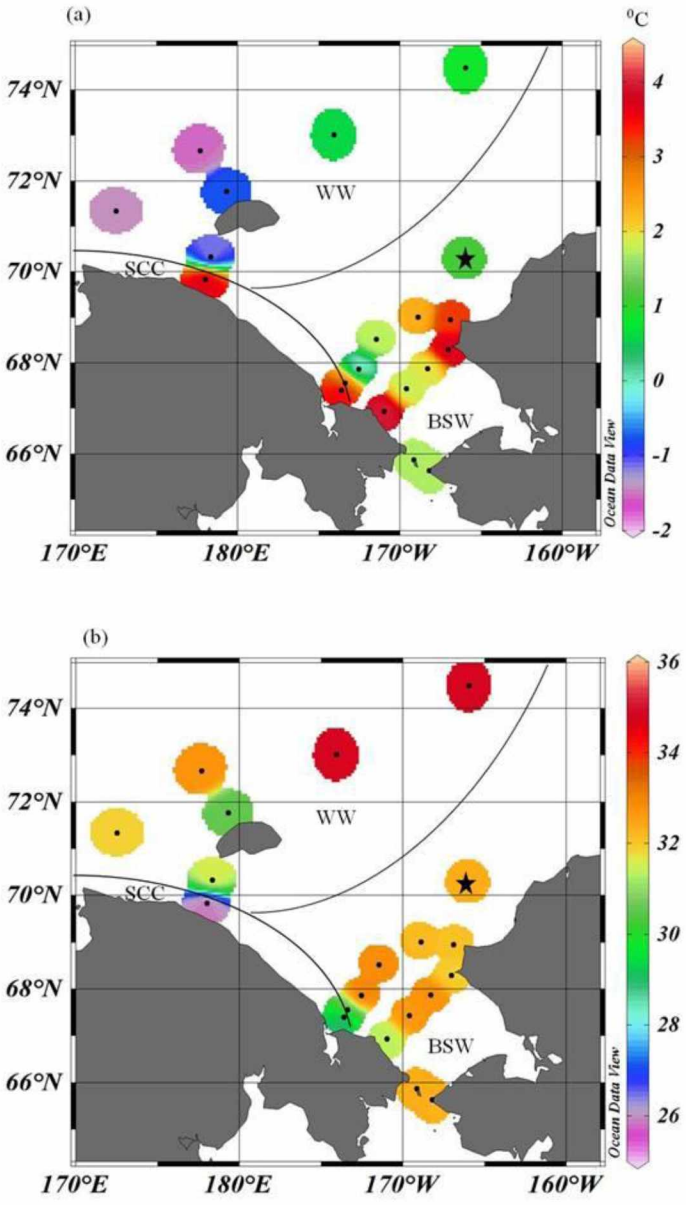

Figure 4. Water temperature (a) and salinity (b) measured in the bottom seawater of the Chukchi Sea on July 30 during COMIDA and September 3 to 30 during RUSALCA research cruises in 2009. RUSALCA stations are displayed as the black dots and the COMIDA station is the black star. Bering Sea Water (BSW), Winter Water (WW) and Siberian Coastal Current (SCC) are the bottom water masses and are divided by the black lines.

was twice as concentrated as in bottom seawater (Figure 5). In the northern Chukchi Sea, Winter Water (WW) spread across the continental shelf and into the Arctic Ocean basin with varying bottom water depths, ranging from 31.7 to 347.0 m. The WW was the coldest (ranging -1.5 to 0.8 °C; Figure 4a) and most saline (ranging 31.7 to 34.8; Figure 4b) water mass. Conversely to BSW trends, Ba in WW sediment interface seawater was about three times lower than in bottom seawater (Figure 5). Compared to the other bottom water masses, Siberian Coastal Current (SCC) was shallow and nearshore to the northeastern coast of Siberia. The SCC was the least saline (ranging 25.8 to 28.7), but warmest (mean of 3.7 °C) water mass (Figure 4).

3.2 Inter- and intraspecific species

The general trends of the elemental pathway via bottom seawater, whole fish blood, and into the otoliths were unique to each element but similar for all three Arctic fish species (Figure 6). The Mg/Ca ratio was highest in the seawater, decreased slightly in the whole blood while remaining in the same order of magnitude, and was about four orders of magnitude lower in the otoliths. The Sr/Ca ratio was highest in the bottom seawater and whole blood, and then decreased by about one order of magnitude as it was incorporated into the otoliths. The Ba/Ca ratio in bottom seawater and otoliths had similar orders of

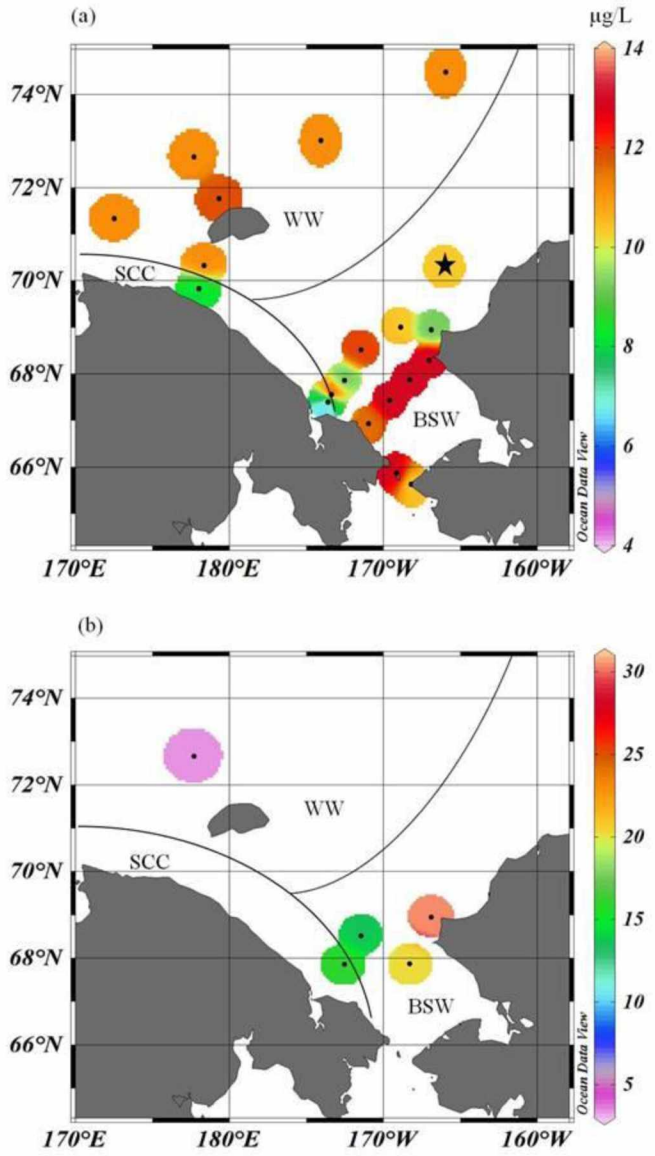

Figure 5. Barium concentrations (µg/L) in bottom seawater (a) at 20 stations collected on July 30 during COMIDA and September 3 to 30 during RUSALCA research cruises in 2009 and Ba concentrations in sediment interface seawater (b) at 5 stations during RUSALCA in 2009 in the Chukchi Sea. The COMIDA station is displayed as the black star and RUSALCA stations are the black dots. Bering Sea Water (BSW), Winter Water (WW) and Siberian Coastal Current (SCC) are the bottom water masses and are divided by the black lines.

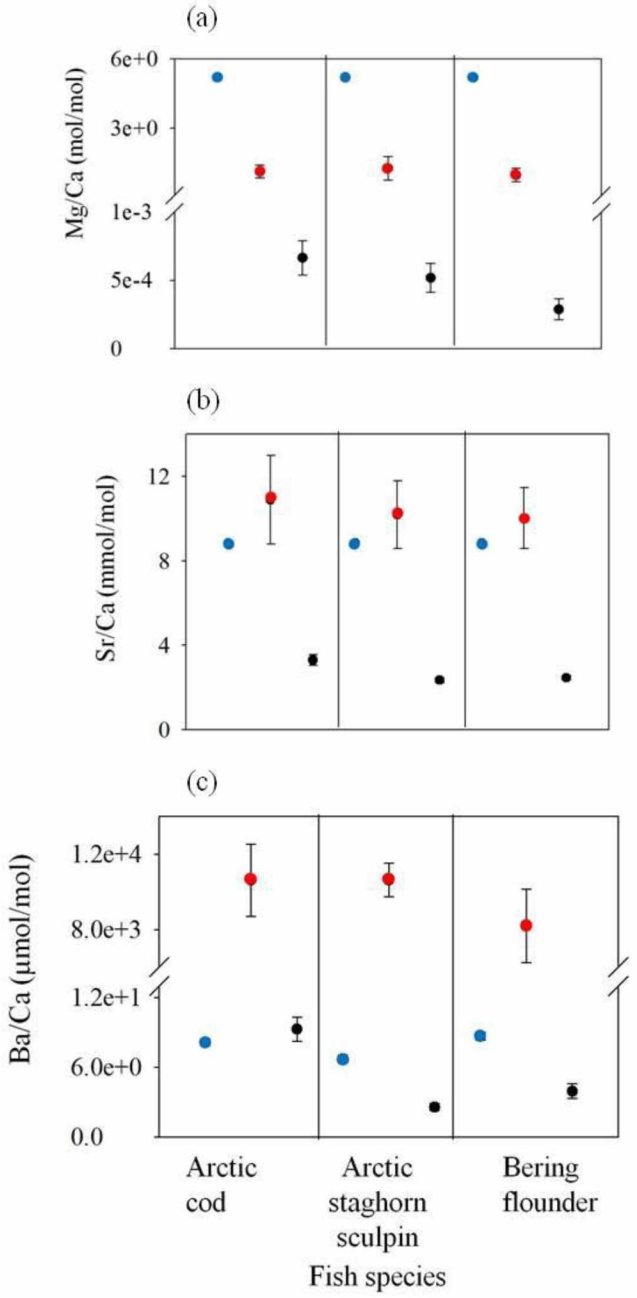

Figure 6. Mg/Ca (a), Sr/Ca (b), and Ba/Ca (c) mean ratios and standard errors in bottom seawater, whole fish blood, and otoliths of three Arctic marine fish species. Vertical black lines divide Arctic cod (left), Arctic staghorn sculpin (center), and Bering flounder (right) in all graphs. Means for elemental ratios in bottom seawater (blue circles), whole fish blood (red circles), and otoliths (black circles) are presented along with 2 standard errors (black bars).

magnitude while whole blood was about three to four orders of magnitude higher. The three demersal species captured in the BSW (Figure 3) showed varying means and ranges for Mg/Ca, Sr/Ca, and Ba/Ca ratios on the otolith edge (Table 2). Arctic cod had the highest means and widest ranges of all elements among fish species. Arctic staghorn sculpin had the lowest means of Sr/Ca and Ba/Ca and had the narrowest range of Ba/Ca among fish species. Bering flounder had the lowest mean for Mg/Ca and narrowest ranges for Mg/Ca and Sr/Ca.

Otoliths were chemically distinct for Mg/Ca and Ba/Ca but not for Sr/Ca in the BSW for all three Arctic fish species. The Mg/Ca ratio was different among species (ANOVA: $F_{2, 203} = 14.97, p < 0.001$), and Mg/Ca in otoliths of Arctic cod was different from Arctic staghorn sculpin (Tukey: $p < 0.001$) and Bering flounder (Tukey: $p < 0.001$). The Sr/Ca ratio showed no significant differences among species (ANOVA: $F_{2, 198} = 0.37, p = 0.69$). The Ba/Ca ratio in the otolith edge was different among (ANOVA: $F_{2, 199} = 71.94, p < 0.001$) and between all fish species (Tukey: $p < 0.001$).

Chemical signatures of the otolith edge differed among water masses for Arctic cod, but not for Arctic staghorn sculpin and Bering flounder. Arctic cod were captured in all three water masses (Figure 3) and differed in Mg/Ca (ANOVA: $F_{2, 125} = 7.97, p < 0.001$), Sr/Ca (ANOVA: $F_{2, 126} = 55.89, p < 0.001$), and Ba/Ca (ANOVA: $F_{2, 124} = 11.22, p < 0.001$) among water masses. Arctic cod collected in BSW had higher Mg/Ca in the otoliths than in WW (Tukey: $p < 0.001$) (Table 2). The Mg/Ca ratio in Arctic cod otoliths from SCC

Table 2

Means and ranges of otolith element/calcium ratios for Arctic fishes collected in Chukchi Sea bottom water masses. Bottom water masses include Bering Sea Water (BSW), Siberian Coastal Current (SCC), and Winter Water (WW). Mean values of elemental ratios are bolded followed by sample size in parentheses. Ranges are listed below mean values in parentheses. Otolith chemistry data, not $\log_{10}$ transformed.

Water mass by species	Otolith chemistry ratios		
	Mg/Ca [mmol/mol]	Sr/Ca [mmol/mol]	Ba/Ca [μmol/mol]
Arctic cod			
BSW	**0.80** (68)	**2.62** (68)	**7.86** (66)
	(0.04-3.02)	(1.29-5.63)	(1.27-25.59)
SCC	**0.48** (15)	**2.47** (15)	**7.16** (15)
	(0.03-1.24)	(1.40-3.99)	(1.00-17.69)
WW	**0.51** (43)	**4.64** (44)	**12.16** (44)
	(0.02-2.95)	(2.38-6.90)	(4.08-29.26)
Arctic staghorn sculpin			
BSW	**0.53** (78)	**2.31** (78)	**2.92** (76)
	(0.02-2.62)	(1.10-4.26)	(0.47-9.29)
SCC	**0.50** (36)	**2.49** (35)	**2.76** (36)
	(0.02-1.68)	(1.06-4.16)	(0.55-5.16)
Bering flounder			
BSW	**0.29** (59)	**2.46** (56)	**3.94** (60)
	(0.01-1.33)	(1.84-3.50)	(0.61-9.59)

water mass was not different from BSW (Tukey: $p = 0.22$) and WW (Tukey: $p = 0.58$). Arctic cod otoliths from WW had higher Ba/Ca than BSW (Tukey: $p < 0.001$) and SCC (Tukey: $p = 0.002$) and higher Sr/Ca than BSW and SCC (Tukey: $p < 0.001$). Arctic cod were significantly older in the WW than BSW (Tukey: $p = 0.002$) while ages of fishes in SCC were not different from WW (Tukey: $p = 0.96$) and BSW (Tukey: $p = 0.11$). Arctic staghorn sculpin were collected from two water masses (Figure 3); BSW and SCC, but showed no otolith chemistry differences among water masses for any element ($p > 0.05$). Bering flounder were only collected from BSW (Figure 3) due to sampling limitations; therefore, water mass comparisons could not be made.

3.3 Physical environmental variables

Water temperature and Ba in sediment interface seawater were correlated to otolith chemistry of some species (Table 3), while salinity and Ba in bottom seawater were not correlated to the otolith chemistry of any fish species. Arctic cod Sr/Ca ratios (Figure 7a; Table 3) showed a strong, negative correlation to temperature while Ba/Ca had a moderate correlation (Figure 7b; Table 3). Bering flounder Mg/Ca otolith chemistry was the only circumstance of any species where Mg/Ca was correlated to temperature and showed a moderate, negative correlation (Figure 8; Table 3). In multiple linear regression models, salinity was not significant for any species or element ($p > 0.05$). Ambient bottom seawater Ba/Ca chemistry did not correlate with the Ba/Ca ratio on the otolith edge of any fish species ($p > 0.05$). Arctic cod Ba/Ca otolith chemistry did not

Table 3

Significant multiple and simple linear regression models and associated parameters for Arctic fishes otolith edge chemistry with physical environmental and biological variables. Otolith chemistry data were $\log_{10}$ transformed for statistical analysis. Simple linear regression results are reported below unless indicated by a * = multiple linear regression results for partial correlation, adjusted R^2, and model statistics. Iw = interface water. Arctic fishes are abbreviated as cod = Arctic cod, sculpin = Arctic staghorn sculpin, and flounder = Bering flounder.

Species	Element ratio	Variable	n	r	R^2	F	Coefficient	Intercept	P
cod	Mg/Ca	Age	126	-0.69	0.48	113.73	-0.33	-0.07	< 0.001
	Sr/Ca*	Age	127	0.40	0.49	61.83	0.05	0.51	< 0.001
		Water temperature	127	-0.65	0.49	61.83	-0.06	0.51	< 0.001
	Ba/Ca	Water temperature	125	-0.33	0.11	14.74	-0.05	0.95	< 0.001
sculpin	Mg/Ca	Age	114	-0.71	0.50	112.52	-0.22	-0.09	< 0.001
	Sr/Ca	Age	113	0.36	0.13	16.56	0.03	0.30	< 0.001
	Ba/Ca	Ba Iw	30	0.59	0.35	15.27	0.02	-0.10	< 0.001
flounder	Mg/Ca	Water temperature	59	-0.41	0.17	11.75	-0.13	-0.55	0.001
	Ba/Ca	Ba Iw	44	-0.38	0.14	6.90	-0.04	1.15	0.012

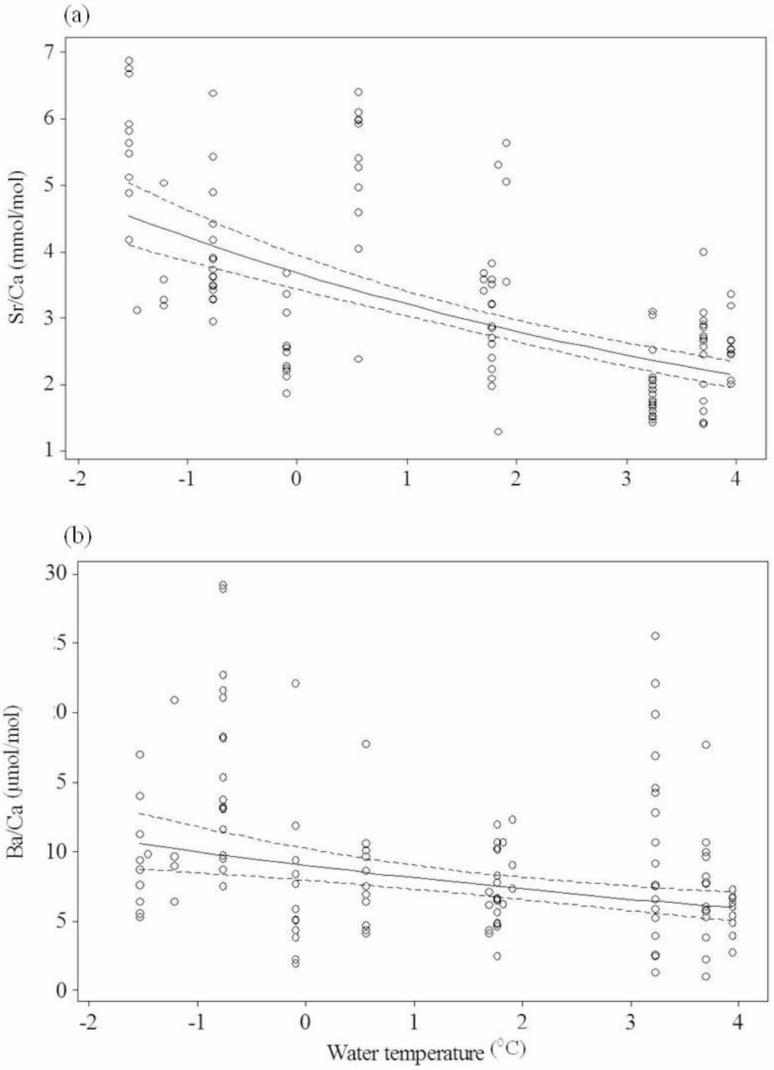

Figure 7. Fitted line plots of Sr/Ca (a) and Ba/Ca (b) on the otolith edge versus water temperature of Arctic cod. Significant linear regressions (solid line) and 95% confidence intervals of the regressions (dashed lines) are displayed.

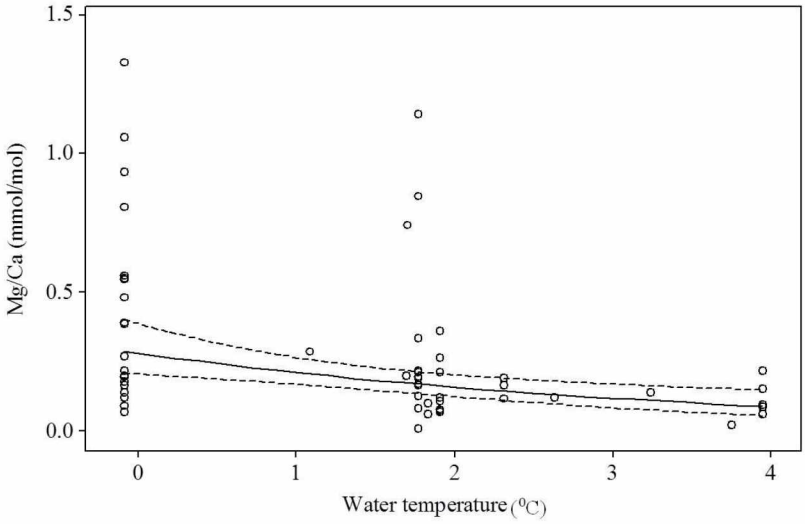

Figure 8. Fitted line plot of Mg/Ca on the otolith edge versus water temperature of Bering flounder. Significant linear regression (solid line) and 95% confidence interval of the regression (dashed lines) is displayed.

have a significant correlation to Ba in sediment interface seawater ($p = 0.95$) while Arctic staghorn sculpin and Bering flounder had significant correlations (Figure 9; Table 3). Arctic staghorn sculpin Ba/Ca showed a strong, positive correlation to Ba in sediment interface seawater while Bering flounder Ba/Ca had a moderate, negative correlation (Figure 9; Table 3). At two stations where Arctic staghorn sculpin and Bering flounder co-occurred and sediment interface seawater was 13.7 and 15.8 µg/L Ba, Arctic staghorn sculpin had about two times lower Ba/Ca ratios in the otoliths ($n = 6$, 2.4 and $n = 14$, 2.0 µmol/mol Ba, respectively) than Bering flounder ($n = 12$, 4.6 and $n = 23$, 4.7 µmol/mol Ba, respectively).

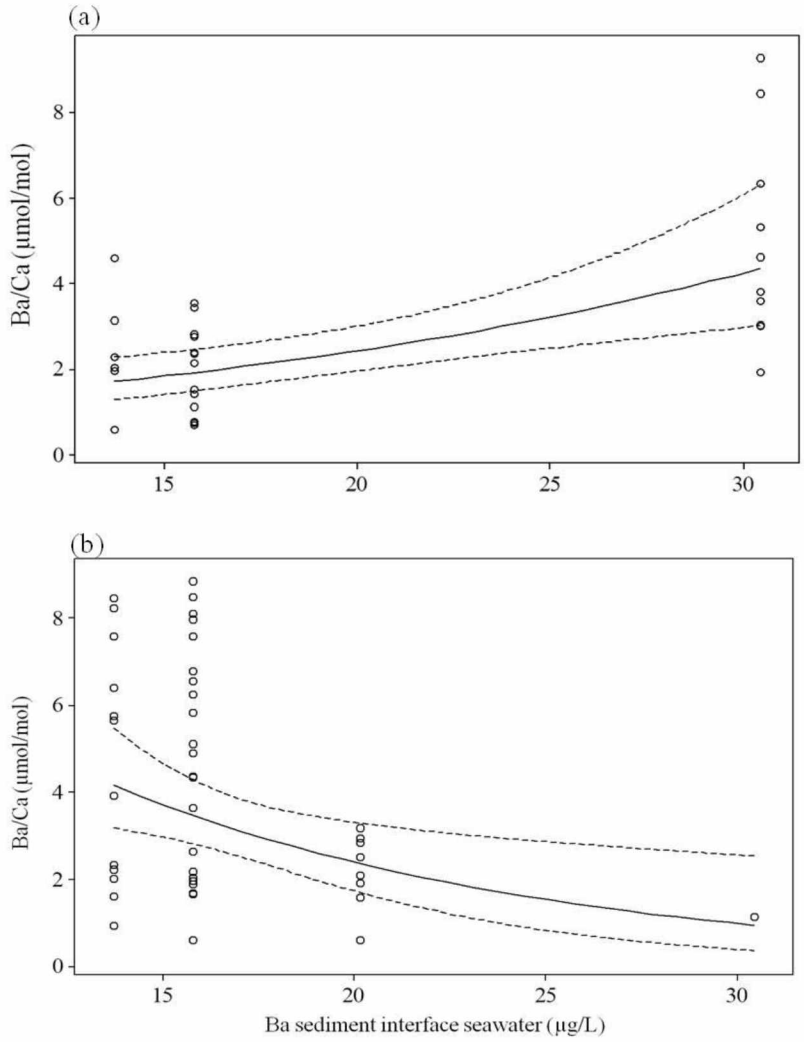

Figure 9. Fitted line plots of Ba/Ca on the otolith edge versus Ba sediment interface seawater of Arctic staghorn sculpin (a) and Bering flounder (b). Significant linear regressions (solid line) and 95% confidence intervals of the regressions (dashed lines) are displayed.

3.4 Biological and physiological variables

Ages of the Arctic demersal fishes correlated to the otolith edge chemistry, while whole blood was not significantly correlated (Tables 3, B-1). Arctic cod ages ranged from 0 to 3 while Arctic staghorn sculpin ranged from 0 to 6 years old (Table 4). Bering flounder

Table 4

Sample sizes of age classes of Arctic fish species separated by bottom water masses collected near the seafloor at 20 stations in the Chukchi Sea. Bottom water masses include Bering Sea Water (BSW), Siberian Coastal Current (SCC), and Winter Water (WW).

	Age (n)									
Species by water masses	0	1	2	3	4	5	6	7	8	total (n)
BSW										**208**
Arctic cod	41	7	17	4	0	0	0	0	0	69
Arctic staghorn sculpin	24	1	28	11	12	2	1	0	0	79
Bering flounder	0	1	19	28	3	5	2	1	1	60
SCC										**51**
Arctic cod	2	7	5	1	0	0	0	0	0	15
Arctic staghorn sculpin	12	0	3	14	7	0	0	0	0	36
WW										
Arctic cod	11	9	19	5	0	0	0	0	0	**44**

ages ranged from 1 to 8 years old (Table 4), but age was not correlated with any element in Bering flounder otoliths ($p > 0.05$). Arctic cod and Arctic staghorn sculpin had strong, negative correlations of Mg/Ca to age (Figure 10; Table 3). Age had an opposite directional effect on Sr/Ca compared to Mg/Ca for both species and showed moderate,

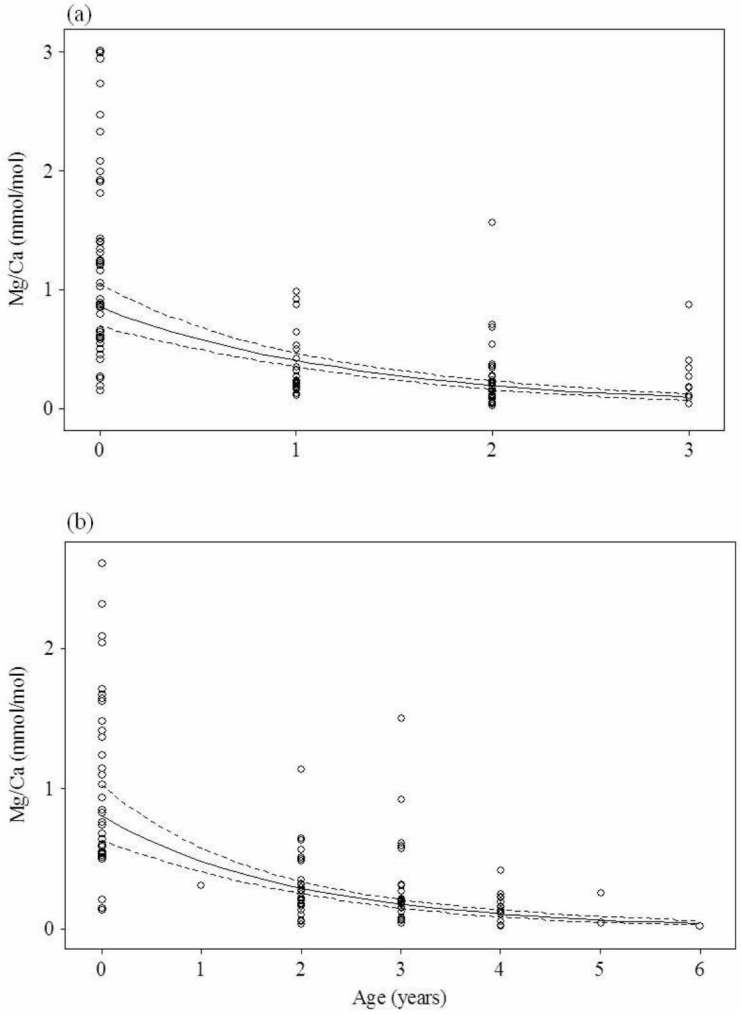

Figure 10. Fitted line plots of Mg/Ca on the otolith edge versus age of Arctic cod (a) and Arctic staghorn sculpin (b). Significant linear regressions (solid line) and 95% confidence intervals of the regressions (dashed lines) are displayed.

positive correlations for Arctic cod and Arctic staghorn sculpin (Figure 11; Table 3). Age-0 Arctic cod and Arctic staghorn sculpin had higher mean values for Mg/Ca and lower Sr/Ca compared to fishes older than age-1. Removing age-0 otoliths from the

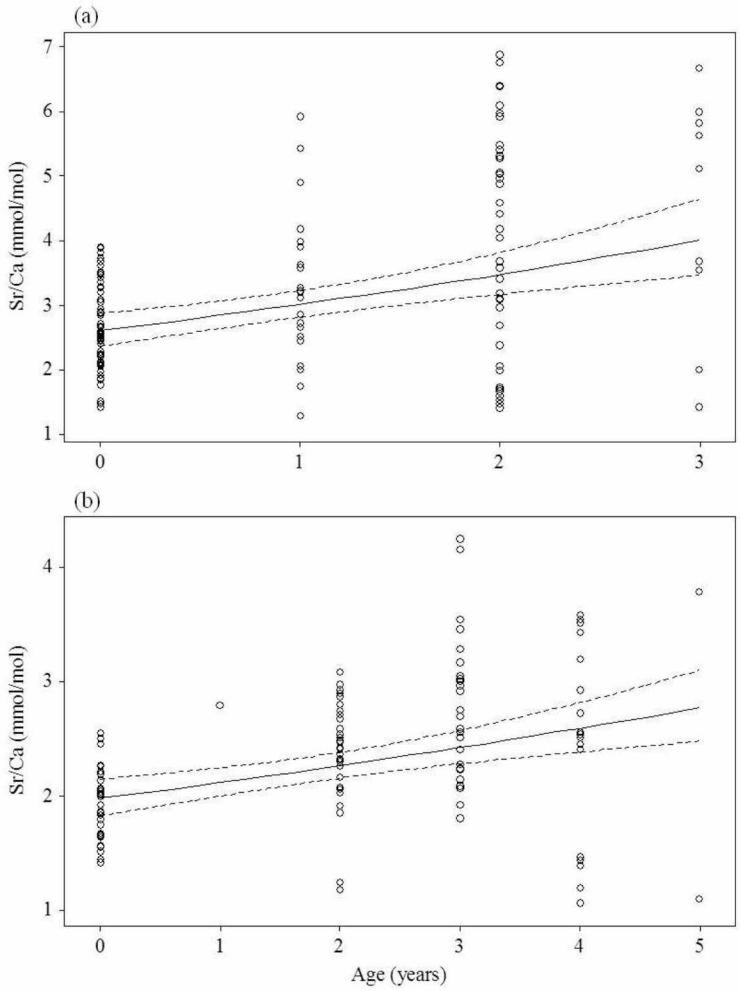

Figure 11. Fitted line plots of Sr/Ca on the otolith edge versus age of Arctic cod (a) and Arctic staghorn sculpin (b). Significant linear regressions (solid line) and 95% confidence intervals of the regressions (dashed lines) are displayed.

linear regressions and retaining fishes older than age-1 of both species in the model for Mg/ Ca and Sr/Ca, yielded only significant negative correlations for Mg/Ca (Arctic cod: $n = 74$, $r = -0.27$, $p = 0.02$; Arctic staghorn sculpin: $n = 78$, $r = -0.40$, $p < 0.001$). Age was not correlated to Ba/Ca in otoliths for the three Arctic fish species ($p > 0.05$).

4. Discussion

The incorporation of ambient trace metals in seawater into otoliths of demersal fishes is not simply related to one category of physical environmental, biological, or physiological parameters. Instead it is a complex mixture of all parameters for Arctic cod, Arctic staghorn sculpin, and Bering flounder in the Chukchi Sea. The goal of this study was to determine if elemental uptake along the recent growth edge of otoliths in Arctic demersal fishes is correlated with physical environmental, biological, or physiological factors. Fish species and age were the primary biological factors that correlated to otolith chemistry of these fishes while water temperature and barium (Ba) concentrations in sediment interface seawater were the main environmental factors. This was the first otolith chemistry study to investigate correlations of Ba concentrations in sediment interface seawater and whole blood chemistry to otolith chemistry of Arctic marine fishes. Values of Ba/Ca in otoliths should be interpreted cautiously as the quality control of percent relative error in the standard reference material FEBs-1 (certified chemical values in homogenous otolith) was approaching 20% in this study. Individual elemental signatures in otoliths did not exhibit the same correlations among species or factor of

interest, which emphasizes the importance of this fundamental study prior to addressing fisheries research questions with otolith chemistry.

Age effects on strontium (Sr) to calcium (Ca) ratios in the otoliths of the three Arctic fish species can be linked to the logarithmic growth trends of fishes. Ages of fishes can influence Sr/Ca in otoliths of wild marine fishes (Sadovy and Severin 1994). In the otolith of an Arctic staghorn sculpin, the ratio of Sr/Ca increases over a life history transect from the core to the edge (Brown and Severin 2009). A more subtle increase in Sr/Ca is noticeable in Bering flounder transects (Brown and Severin 2009). The strength of these trends in these two fish species parallels the outcomes of this study. An increase in growth rate in otoliths (Sadovy and Severin 1994) and corals (Sinclair 2005) reduces the entrainment of Sr into the aragonite structures. As the age of fish increases, the protein synthesis and otolith growth decreases (Schwarcz et al. 1998, Campana 1999). Metabolic rates are correlated with otolith accretion rates (Wright 1991). Body growth rate and Sr/Ca in otoliths are inversely correlated in fish species from different localities (Sadovy and Severin 1994), though the relationship may not hold for true for all fish species (Kalish 1989, Gallahar and Kingsford 1992).

The correlations of age to magnesium (Mg)/Ca and Sr/Ca in otoliths of Arctic cod and Arctic staghorn sculpin are likely attributable to ionic effects of the incorporation of elements into aragonite crystal lattices during low and high growth periods of fishes (Sinclair 2005). In this study, fishes were collected during the seasonally high growth

period of these Arctic fishes and the recent growth edge of the otolith was sampled in the region of high calcification as indicated by wide growth bands. The ratios Mg/Ca and Sr/Ca in aragonite corals and otoliths are correlated to calcification rates (Sinclair 2005, Hamer and Jenkins 2007). Due to the similar sized ionic radius of Sr and Ca atoms, periods of high calcification rates would lead to a dilution of Sr in the otolith (Campana 1999, Sinclair 2005, Hamer and Jenkins 2007). The incorporation of Mg into otoliths is not well understood, but the causality of Mg correlations to accretion rates in otoliths may be similar to that of coral aragonite. The uptake of Mg in coral aragonite is positively correlated to calcification rates due to the higher occurrence of crystal defect sites during fast growth periods, where Mg would be trapped during periods of high calcification (Sinclair 2005). Crystallization processes of the otolith are likely controlled by the formation rate of the protein matrix on the growing otolith surface, which in turn is highly correlated with somatic growth rate, metabolic rate, and water temperature (Campana 1999).

For Arctic cod and Arctic staghorn sculpin, age-0 fishes played a pivotal role in the observed, significant correlations between age and Sr/Ca otolith chemistry, whereas Mg/Ca was unaffected by the inclusion of this age class. Overlap of Sr/Ca in otoliths exists in fishes older than age-1, which indicates age is not necessarily a reliable predictor for Sr/Ca trends in Arctic fish otoliths. Trends of Mg/Ca and Sr/Ca in the otoliths of these two species may be explained by the ionic effects on the elemental incorporation into the otolith during high growth periods. Alternatively, young fishes have immature

osmoregulation in ambient seawater allowing more ions to enter the body that in turn are available for otolith accretion (Kalish 1991, Fowler et al. 1995). As fishes develop, the ability to discriminate ambient elements from seawater improves. The Mg/Ca ratio is an order of magnitude higher in bottom seawater than Sr/Ca (Riley and Chester 1971, Libes 2009), for which we would expect both elements in the otoliths to be higher in age-0 than older fishes due to immature osmoregulation. However, the osmoregulation argument for young fishes was not supported by the results of this study because Sr/Ca was lower and Mg/Ca was higher in age-0 fish otoliths compared to older fishes.

This study confirmed water temperature effects on otolith chemistry in field-collected Arctic fishes of various ages. For temperature, or any physical environmental variable, to influence the otolith chemistry of demersal fishes, the otolith material analyzed must have been accreted while the fishes inhabited a particular temperature regime in bottom seawater. Laboratory experiments controlling these parameters within ranges observed in the natural environment are needed to validate this assumption for Arctic cod, Arctic staghorn sculpin, and Bering flounder. The ratios of Sr/Ca and Ba/Ca on the otolith edge are inversely correlated to temperature in wild Arctic cod in this study and cold-adapted (2, 5, and 8 ^{o}C) larval Pacific cod, *Gadus macrocephalus*, reared under laboratory conditions while no effect was found for Mg/Ca in either study (DiMaria et al. 2010). Negative temperature effects on Sr/Ca in otoliths have mainly been documented in cold-adapted fish larvae (*Gadus morhua* and *Clupea harengus*) reared in the laboratory (Radtke et al. 1990, Townsend et al. 1992, 1995). Congruent to the results of Arctic cod

and Arctic staghorn sculpin, temperature often has no correlation with Mg/Ca in otoliths of larval and juvenile marine fishes (Hoff and Fuiman 1995, Martin and Thorrold 2005, DiMaria et al. 2010) while evidence exists for inverse effects of juvenile Atlantic croaker (*Micropogonias undulatus*) (Fowler et al. 1995). Laboratory experiments that explore the influence of abiotic factors on otolith chemistry are feasible for larval and juvenile fishes whereas an effect of age class is hard to discern without field-collected fishes.

As fishes experience cool seawater temperatures, Sr/Ca and possibly Ba/Ca should increase in otoliths due to decreased somatic growth (Sadovy and Severin 1992, 1994) and lower metabolic rates of fishes (Radtke 1989, Townsend et al. 1992, 1995), while Mg is likely tightly regulated at these temperatures (DiMaria et al. 2010). In this study, the negative correlation between Sr/Ca in otoliths of Arctic cod and temperature may be explained by the ionic effects of Sr substitution in otoliths due to decreased growth (DiMaria et al. 2010). The inverse trend of Sr/Ca and temperature has also been explained by the effects of temperature on the kinetic energy of dissolved ions and diffusion rate into aragonite (Kinsman and Holland 1969, Campana 1999, Brown and Severin 2009). It is difficult to determine the causality of the effect of temperature on Ba because this element is known to both substitute directly for Ca, like Sr (Speer 1983, deVries et al. 2005), and attach at crystal lattice defect sites, like Mg (Lea and Spero 1992, Campana 1999). Assuming that Ba attached at the crystal lattice defect sites, Mg/Ca and Ba/Ca in otoliths were probably not a result of ionic effects because these two elements would have had positive correlations to temperature, which was not the case in

this study. Instead, Ba probably substituted directly for Ca in Arctic cod otoliths. Magnesium is more important for cellular processes and may be more tightly regulated in fishes than Sr and Ba; therefore, temperature may not influence the otolith deposition of this element for all fish species (DiMaria et al. 2010). Temperature may not directly influence otolith chemistry, but instead be a cue for the onset of seasonal changes in marine fish physiology that alter the available Sr ions for otolith formation (Kalish 1991).

Marine fishes inhabiting a wide range of water temperatures are more likely to exhibit a temperature effect on otolith chemistry (Campana 1999). In order for otolith chemistry to be a valuable tool for reconstruction of water temperature regimes, fish living in water colder than 10 °C would need to experience at least a 15 °C ambient temperature difference to influence Sr/Ca in an otolith as drastic as the effects of salinity on otolith chemistry in anadromous fishes (Campana 1999). Correlations between temperature and otolith concentrations of Sr and Ba in Arctic cod otoliths existed because this species experienced the highest range of ambient seawater temperatures and was present in all three water masses in the Chukchi Sea. Temperature did not correlate with Sr/Ca in Arctic staghorn sculpin otoliths probably because the distribution of this species was limited to Bering Sea Water (BSW) and Siberian Coastal Current (SCC). Likewise, Bering flounder was only captured in BSW in the southern Chukchi Sea and thus they experienced only a limited range in bottom water temperatures.

Differences of Ba/Ca in the otolith edge of the three demersal fish species were probably attributed to species-specific diets. In this study of Arctic marine fishes and in another study involving freshwater lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) (Melancon et al. 2009), Ba is elevated in the whole blood compared to seawater and otoliths, which could mean there is an alternative source of Ba besides ambient seawater. Prey composition and elevated levels of Ba measured in prey affect Ba/Ca otolith chemistry (Gallahar and Kingsford 1996, Buckel et al. 2004). Diets of all three fishes have been assessed off the northwestern coast of Alaska in the Chukchi Sea from August to September 1990 and 1991 (Coyle et al. 1997). Arctic cod prey on planktonic and epibenthic organisms, including Bering flounder. Arctic staghorn sculpin have been found as prey in Arctic cod and Bering flounder. Bering flounder primarily consume fishes and crustaceans while Arctic staghorn sculpin prefer polychaetes and mollusks. The highest to lowest mean Ba/Ca in the otoliths by fish species was Arctic cod > Bering flounder > Arctic staghorn sculpin. This ordered trend in otolith Ba/Ca may correspond to the diets of these three demersal species and explain interspecific species differences in otolith chemistry. In future studies, chemical composition of prey may provide better evidence for diet effects on Ba/Ca in otoliths.

Alternatively, differences in Ba/Ca in the otoliths of the three species in BSW may be related to habitat based on inferences of body morphology and distribution within the water column. Arctic cod had the highest Ba/Ca in the otoliths compared to Arctic staghorn sculpin and Bering flounder maybe because this species has been known to

occupy the most diverse range of habitats in the Chukchi Sea, i.e., demersal, upper water column, and river mouths (Craig et al.1982, Moline et al. 2008), where the latter two habitats are expected to have the highest concentrations of dissolved Ba in the water (Guay and Falkner 1997). Arctic staghorn sculpin and Bering flounder have different site fidelity to habitats compared to Arctic cod because they primarily live near and half buried within the seafloor sediments from juvenile to adult life stages (Mecklenburg et al. 2002), yet differences in Ba/Ca in the otoliths between these two species may be further explained by their proximity to the seafloor sediments. In BSW, Arctic staghorn sculpin had the lowest Ba/Ca in the otoliths of the three fish species and was twice as low compared to Bering flounder at two stations where these species co-occurred and Ba in sediment interface seawater was measured. The body morphology of the Bering flounder is designed for them to rest and be buried in sediment, while Arctic staghorn sculpin are likely to be more mobile near the seafloor. The contrasting positions near the seafloor may explain why Bering flounder have higher concentrations of Ba in the otoliths than Arctic staghorn sculpin at the same stations.

The opposing correlations between otolith Ba/Ca in Arctic staghorn sculpin and in Bering flounder with Ba concentrations in sediment interface seawater could have been a result of species-specific dietary differences and tight benthic-pelagic coupling. Both species reside in demersal habitats and have overlapping geographical distributions in the Chukchi Sea (Mecklenburg et al. 2002, 2007). Elevated levels of Ba in bottom seawater and seafloor sediment are associated with high export of particulate Ba from high

productivity areas in overlying surface waters in the Chukchi Sea (Guay and Falkner 1997, Taylor et al. 2003, Abrahamsen et al. 2009). High productivity of shallow shelf seas, such as the Chukchi Sea, result in tight benthic-pelagic coupling (Dunton et al. 2005). The Chukchi Sea has areas with high benthic biomass known as "hotspots", which are supported by high phytoplankton biomass exported from the overlying water column (Grebmeier et al. 1988, Dunton et al. 2005). This patchiness in benthic biomass could then account for the diverse range of Ba measured in sediment interface seawater. Overlying flow of ocean currents directly influences the benthic community structure, carbon export, and sediment composition (Grebmeier and Dunton 2000). Areas in the Chukchi Sea differing in benthic biomass may reflect specific Ba sediment signatures and have varying levels of Ba entrained into benthic species that scavenge on exported particulate Ba from overlying productive waters. Arctic cod may not be as tightly coupled to benthic prey compared to Arctic staghorn sculpin and Bering flounder as this species often occupies the pelagic and cryopelagic realms where it feeds on planktonic and ice-associated organisms (Craig et al.1982, Moline et al. 2008).

Otolith chemistry laboratory and field studies have mixed results on the extent to which diet influences the incorporation of Ba/Ca into marine fish otoliths. Concentrations of Ba in otoliths of laboratory-reared tropical damselfish (*Acanthochromis polyacanthus*) are influenced by food rations, temperature, and life history stages (Walther et al. 2010). Diet composition of natural prey of young-of-the-year bluefish (*Pomatomus saltatrix*) altered the concentration of Ba/Ca in the otoliths whereas the quantity of prey did not

have an effect in a laboratory study (Buckel et al. 2004). Ambient seawater concentrations of Ba have contributed to 98% of the Ba/Ca chemical signature in otoliths compared to artificial food at 2% in laboratory-reared juvenile mummichogs (*Fundulus heteroclitus*) based on an isotope mixing model of Ba^{138}/Ba^{137} (Walther and Thorrold 2006). In a field study, species-specific diets likely did not cause the Ba/Ca differences in otoliths among juvenile demersal fishes, the silver seabream (*Pagrus auratus*) and the sand flathead (*Platycephalus bassensis*), because they have similar diets of crustaceans and fishes (Hamer and Jenkins 2007). Contributions of prey should be considered when determining the causality of Ba/Ca in otoliths of marine species (Buckel et al. 2004) and may have influenced the results in the current study.

Chemical concentrations of Ba in ambient seawater may influence otolith chemistry in marine fishes if they experience a wide range of Ba concentrations in water. In this study, ambient bottom seawater had a small range of Ba concentrations on a large geographical scale across the Chukchi Sea and may explain the non-existent correlation of Ba/Ca in otoliths of all three species. Compared to Ba/Ca in bottom seawater, exposure to wider ranges of Ba concentrations in sediment interface seawater produced a correlation with Ba/Ca otolith chemistry of Arctic staghorn sculpin and Bering flounder, but not Arctic cod. Laboratory-reared marine fishes exhibiting correlations between Ba/Ca in otolith chemistry and ambient seawater often experienced a wider range of Ba/Ca in seawater above natural environment ratios (Bath et al. 2000). In contrast to the results of this study, silver seabream and sand flathead are temperate demersal species

that exhibit correlations of Ba/Ca in otoliths and ambient bottom seawater (3.89-11.94 μmol/mol in seawater; Hamer and Jenkins 2007) while inhabiting a similar range of ambient seawater Ba concentrations to this current study (3.99-11.37 μmol/mol). However, the correlations of otolith chemistry and ambient seawater may not be transferrable between biomes and species because Ba/Ca in otoliths is not solely correlated to seawater concentrations and can be confounded by interactions with other parameters, e.g., diet, water temperature, etc.

Concentrations of Mg and Sr in otoliths of marine fishes are more likely to be influenced by biological and physiological than environmental variables because these elements are conservative in seawater (Campana 1999, Brown and Severin 2009). Salinity had no correlation with otolith chemistry of Arctic fishes; therefore, no correlation would exist to Mg/Ca and Sr/Ca in ambient seawater. Similarly, in temperate bottom seawater in Australia, no significant correlations existed between Mg/Ca and Sr/Ca in bottom seawater and otoliths in demersal species of different families due to the low variability in seawater chemical composition (Hamer and Jenkins 2007). For otolith elemental composition to be influenced by water chemistry and salinity, marine fishes must experience a diverse range of these variables similar to that of diadromous fishes (Campana 1999, Brown and Severin 2009). Though seawater is chemically homogeneous for Mg/Ca and Sr/Ca, Mg/Ca in the otolith edge was different among co-occurring fish species in this study and suggests some form of physiological regulation by the fish. Physiological mechanisms may dictate the incorporation of Mg/Ca into

otoliths of different fish species (Hamer and Jenkins 2007) because Mg is a pertinent element in cellular processes and pathways (Watanabe et al. 1997). Alternatively, growth rate differences among species may account for Mg/Ca differences in otoliths from ionic effects (Hamer and Jenkins 2007). The Sr/Ca ratio in the otoliths was similar for all three fish species and is consistent with results of other studies comparing different families of fishes (Dove et al. 1996). Inter- and intraspecific differences in families of fishes may result in differences of Sr/Ca in otoliths (Gillanders and Kingsford 2003, Hamer and Jenkins 2007); therefore, Sr/Ca trends in marine fishes are not uniform. Whole blood concentrations of Mg/Ca and Sr/Ca were not correlated with otolith composition in these Arctic fishes, though it has been noted that Sr/Ca in blood plasma influence otolith chemistry on a seasonal basis in other gadid species (Kalish 1991). Differences of elemental ratios in otoliths among species could be attributed to the type of antifreeze proteins occurring in the blood sera, which differ between Arctic cod and winter flounder (*Pseudopleuronectes americanus*) (Osuga and Feeney 1978). The protein composition of blood plasma fluctuates on a seasonal basis in bearded rock cod (*Pseudophycis barbatus*) and could affect the chemical composition of the endolymph fluid surrounding the otolith and the otolith itself (Kalish 1991). The ratio of Sr/Ca in marine fish otoliths is likely under biological and physiological control due to seasonal fluctuations in blood and endolymph chemistries (Kalish 1991), species-specific effects (Sadovy and Severin 1994, Hamer and Jenkins 2007, Brown and Severin 2009), age class (Sadovy and Severin 1994), and biomes (Sadovy and Severin 1992, 1994).

Differences in Arctic cod otolith elemental concentrations among BSW, Winter Water (WW), and SCC water masses in the Chukchi Sea can be attributed to seawater temperature and fish age. Arctic cod exhibited the highest means and widest ranges of Sr/Ca and Ba/Ca in the otoliths among the three Arctic fish species and was the only species captured in all three water masses. Temperature had inverse effects on the otolith concentrations of Sr/Ca and Ba/Ca in Arctic cod and both elements were significantly higher in the WW compared to BSW and SCC. The WW, in the northern most Chukchi Sea, had the coldest temperature range (-1.5-0.5 ^{o}C) of the three water masses. Sr/Ca and Ba/Ca in Arctic cod otoliths collected in the WW were most likely a result of ionic effects of decreased fish growth rates at colder temperatures. Ages of Arctic cod were older in WW compared to BSW and may explain differences in Sr/Ca concentrations between these water masses because Sr/Ca increases with age of fishes. Dietary differences and movement of Arctic cod among diverse habitats in the water column are additional possibilities to explain differences of Ba/Ca in otoliths.

5. Conclusions

The physical environment correlates with otolith chemistry in some Arctic marine fishes that live in offshore demersal habitats, though a wider range of magnitudes of environmental variables should be sampled to determine abiotic effects on otolith chemistry for all species. Arctic cod was the only species that exhibited differences among water masses in the Chukchi Sea for Mg/Ca, Sr/Ca, and Ba/Ca otolith chemistry.

Cold bottom water temperatures correlated with Sr/Ca and Ba/Ca otolith chemistry in Arctic cod residing in WW, the most northern water mass. Age also correlated with Sr/Ca in Arctic cod otoliths from WW. Temperature correlated with Mg/Ca in the otoliths of Bering flounder, indicating Mg/Ca has the potential to show differences in otoliths among water masses if fish distribution does not limit sampling success in Arctic waters. Arctic staghorn sculpin and Bering flounder exhibited opposite correlations to Ba in sediment interface seawater and Ba/Ca on the otolith edge while bottom seawater concentrations showed no correlation in either species. Sediment interface seawater chemistry may reveal interesting correlations with otolith chemical signatures and these should be explored in future sampling efforts, especially in areas with high and low benthic production. The ratio of Ba/Ca in otoliths of demersal fishes could be an interesting biomarker for fish residence near benthic biomass hotspots in the Chukchi Sea if the correlation of Ba/Ca in otoliths with Ba in sediment interface seawater is investigated further. Correlations of otolith chemistry to salinity were not found in these three species implying conservative elements in seawater are not directly incorporated into otoliths of marine fishes unless sampled over a wider range of salinities where these elements do not behave conservatively, such as river mouths. For each fish species, abiotic factors correlated differently with otolith chemistry; therefore, basic species-specific relationships need to be established to avoid misleading extrapolations of otolith signatures. Future studies in the Chukchi Sea should consider a seasonal sampling design where Arctic fish otoliths and abiotic factors are collected in brackish water and seawater to establish basic correlations of variables over a wider range of magnitudes. The ratio of

Ba/Ca otolith chemistry has the potential to be a useful natural chemical tag to help answer questions about life history movement patterns, spawning location and timing, and stock identification of these Arctic species while Sr/Ca and Mg/Ca are more correlated to the biology and physiology of these fishes.

Components of biology in conjunction with physiological homeostasis and elemental regulation of Arctic marine fishes appear to play a more consistent role than does the physical environment in the entrainment of conservative and non-conservative seawater elements into the otolith edge. Data in this study suggest that the three species have distinct ratios of Mg/Ca and Ba/Ca but not of Sr/Ca on the otolith edge. It is not necessary to include every element in a multi-element approach to address fisheries questions about marine fish species. Ages of fish should be taken into consideration in future studies that investigate Mg and Sr in otoliths of Arctic fishes. Otolith chemistry of Mg/Ca and Sr/Ca in age-0 fishes was particularly interesting because age-0 fishes had different chemical signatures than fishes older than age-1. Further analyses of physiological regulation or ionic effects in growth rates of otoliths could help determine causality of age-effects. Trophic level and species-specific diets may be possible research avenues to pursue in future sampling efforts. Protein composition and presence of antifreeze molecules in Arctic fish blood appears to be different among species and varies seasonally (Osuga and Feeney 1978), and has the potential to influence the metal-binding capacity of the endolymph fluid and otoliths or excretion of trace elements. Based on the current study, otolith chemistry of these Arctic marine fishes does not provide enough predictive power to distinguish water mass occupation nor allows us to

reconstruct life history movement patterns among bottom waters in offshore areas of the Chukchi Sea.

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Appendix A

Preparation and chemical analyses of whole fish blood

Whole blood samples, standards, and blanks were prepared according to a dilute-and-shoot method (McShane et al. 2008) at the UAF Advanced Instrumentation Laboratory. Seronorm™ trace element whole blood L-2 (Barany et al. 1997, de Boer et al. 2004) was used as the standard reference material (SRM) and was stored frozen until analysis. Whole blood fish samples, sample blanks (2% nitric acid), and SRM samples were diluted with a diluents solution for analysis for a final sample volume of 5 mL. The diluents solution consisted of 1% (v/v) trace metal grade tetramethylammonium hyrodxide (TMAH), 0.05% (v/v) Triton X-100, and 0.1% (w/v) ethylenediaminetetraacetic acid (EDTA) disodium salt, and Milli-Q water. All samples were vortexed for 5 min, left overnight at room temperature to homogenize the solution, and then shaken prior to analysis the next day.

A base blood pool was used to matrix match the fish blood samples in the external calibration standards (McShane et al. 2008, Batista et al. 2009). The base blood pool was prepared from whole blood (in sterile plastic 4 mL BD vacutainers® coated with K_2 EDTA whole blood anticoagulant) of subsistence-harvested Chinook salmon (*Oncorhynchus tshawytscha*) and diluted 1/50 with the diluents solution, vortexed for 5 min, and left overnight at room temperature to homogenize. External calibrations made up of Milli-Q water base solutions failed to recover SRM certified concentrations once

fish blood samples and SRM standards were analyzed; therefore, matrix matching was necessary. External calibration stock solutions of calcium (Ca), strontium (Sr), magnesium (Mg), were prepared for 10, 20, 100, 200, 250, and 300 µg/L while barium (Ba) ranged from 1, 2, 10, 20, 25, 30 µg/L based on expected fish blood concentrations from Kalish 1991 and Melancon et al. 2008. Continuous calibration blanks (CCB) consisted of a 1/50 dilution of base pool blood to diluents solution.

$Ca^{42, 43, 44}$, $Mg^{24, 25}$, $Sr^{86, 88}$, and $Ba^{137, 138}$ were analyzed in whole blood samples with an Agilent 7500ce quadrupole inductively coupled plasma mass spectrometer (ICP-MS) (McShane et al. 2008). $Iridium^{193}$ (McShane et al. 2008, Batista et al. 2009) and $Rhodium^{103}$ (de Boer et al. 2004, Batista et al. 2009) were used as internal standards (ISTD). ICP-MS components were cleaned and tubing was replaced after the completion of each analytical day. External calibrations were analyzed at the beginning of the analytical run followed by a set of five blank and fish blood sample pairs, then a SRM, Continuous calibration verification (CCV), and CCB. ICP-MS cones were conditioned with a matrix-matched calibration blank for about 20 min after the external calibration and prior to analysis of fish blood samples. Rinse solutions of 2% nitric acid with 0.002% Triton X-100 and the diluents solution were used after each sample was analyzed. External calibration standards of 20 and 200 µg/L were used as CCVs. The first analytical run's duration was 7 hrs and dilution levels consisted of external calibration standards of 1/50, SRMs 1/50 and 1/200, fish blood 1/50, and sample blanks 1/50. The second run lasted 21 hrs at a higher dilution level of 1/100 for all samples.

Element to Ca ratios were determined by respective blank subtraction and simple linear regression of external calibration (McShane et al. 2008). All sample analytes were acquired in cps for each gas mode; normal (no gas), hydrogen (H), and helium (He). Sample analytes were first divided by Rhodium ISTD cps in the respective gas modes. Blank correction of samples was completed by subtraction of sample blanks from fish blood and SRM samples while calibration blanks were subtracted from calibration standards. External calibration lines were used to determine the simple linear regression equations for each analyte and gas mode. Blank-subtracted fish blood and SRM sample analytes were assessed in the calibration equations for a final concentration in µg/L and multiplied by the appropriate dilution factor. Analyte to Ca ratios were reported for whole blood fish samples to compare to otolith values. Isotopes of each element and ICP-MS gas modes with the most accurate measurement recoveries in the SRMs were reported for element to Ca ratios in fish blood samples. Limits of detection (LOD) for analytes in the SRM were calculated by three times the standard deviation of the sample blanks. The first analytical run had LODs of 114.59 Ca^{44} (H mode), 13.94 Mg^{24} (He mode), 0.80 Sr^{86} (normal mode), and 1.32 µg/L Ba^{137} (normal mode) while the second run had typical values of 93.09 Ca^{44} (H mode), 323.06 Ca^{44} (normal mode), 25.29 Mg^{24} (He mode), 0.529 Sr^{86} (normal mode), and 3.49 µmol Ba^{138} (H mode). Averaged percent relative errors (Equation 5) of SRMs in the first run were 7.6% Mg^{24}/Ca^{44}, 37.1% Sr^{86}/Ca^{44}, and 5.7% Ba^{137}/Ca^{44} while the typical values in the second run were 6% Mg^{24}/Ca^{44}, 11.5% Sr^{86}/Ca^{44}, and 32.7% Ba^{138}/Ca^{44}. LOD and percent relative errors of

the ICP-MS increased over the duration of the analytical runs; therefore, the duration of runs should be limited to less than 10 hrs in future sampling efforts.

Appendix B

Results of elemental ratios in whole blood of Arctic fishes

Elemental ratios of Mg, Sr, and Ba to Ca in whole fish blood and along the recent otolith edge were not correlated in Arctic cod, Arctic staghorn sculpin, and Bering flounder in the Chukchi Sea (Table B-1).

Table B-1.

Arctic cod, Arctic staghorn sculpin, and Bering flounder Spearman correlations coefficients for Mg/Ca, Sr/Ca, and Ba/Ca ratios in whole blood and otoliths. Correlations coefficients are presented in bold, sample sizes are in parentheses, and significance levels are listed below correlation coefficients.

	Fish species		
	Arctic cod	Arctic staghorn sculpin	Bering flounder
	Whole blood		
	Mg/Ca [mol/mol]		
Otolith			
Mg/Ca [mmol/mol]	**0.16** (27) 0.43	**-0.17** (54) 0.23	**-0.37** (27) 0.06
	Sr/Ca [mmol/mol]		
Sr/Ca [mmol/mol]	**0.28** (27) 0.15	**0.17** (53) 0.21	**0.33** (27) 0.10
	Ba/Ca [mmol/mol]		
Ba/Ca [µmol/mol]	**0.08** (27) 0.67	**-0.19** (51) 0.18	**0.39** (27) 0.05

In Bering flounder, Ba/Ca in whole blood and otoliths was borderline significant ($p < 0.05$), though Figure B-1 shows no visible trend in the data.

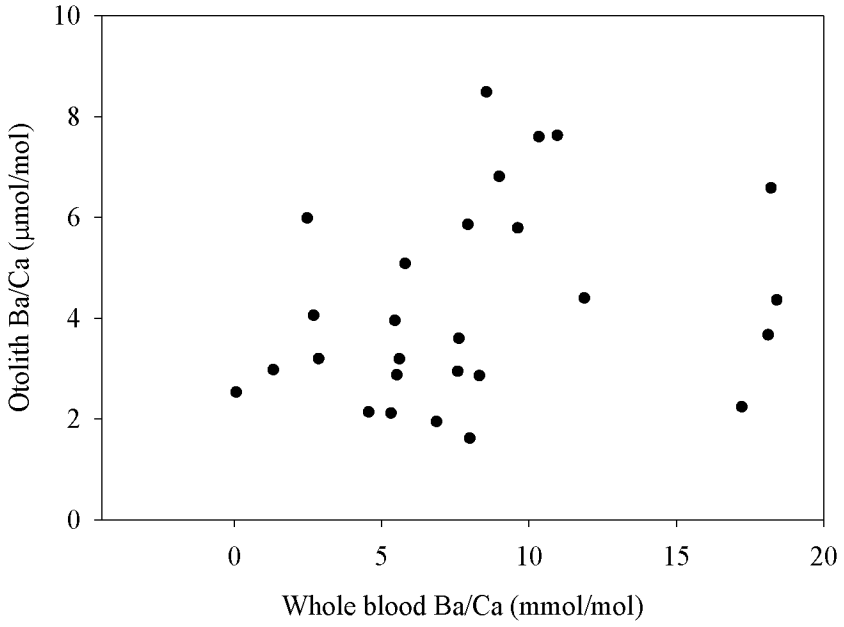

Figure B-1. Scatter plot of Ba/Ca ratios in otoliths and whole fish blood of Bering flounder. Spearman correlation $r = 0.39$, $p = 0.05$.

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